EFFECTS OF GANGLIOSIDE GM_1 ON MDMA-INDUCED SEROTONERGIC NEUROTOXICITY IN THE RAT BRAIN

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by

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An Abstract of a Thesis by
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June 1995
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MDMA (Ecstasy), a methylamphetamine derivative, has been found to produce severe and somewhat selective damage to CNS serotonergic (5-HT) neurons. This damage, though extensive, does not involve cell death. The ganglioside GM_1 has demonstrated potential neurotrophic properties which may enhance the rate of recovery of the neurons from the MDMA-induced effects. To assess this GM_1 effect, MDMA (20 mg/kg x 4 days), GM_1 (40 mg/kg), MDMA and GM_1 or saline was injected (i.p.) into male rats. Behavioral activity was determined for 24 hours on day 5 or day 12 following the beginning of the injections. A general increase in activity on day 5 was noted for the MDMA group (consistent with a release of 5-HT and/or catecholamines) while the activity of the MDMA/GM_1 group was lower, at the level of the control. At day 12 the MDMA group's activity was decreased relative to the control (consistent with a 5-HT depletion) while the MDMA/GM_1 group's activity was at or above that of the control. Biochemical analysis of brain tissue obtained from the animals sacrificed on day 7 or 14 following the beginning of the injections demonstrated a profound depletion of 5-HT, dopamine and norepinephrine with administration of MDMA. The MDMA/GM_1 group also showed this depletion, though to a smaller degree, suggesting some attenuation of toxic effects.

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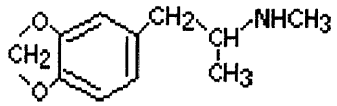
INTRODUCTION AND REVIEW OF THE LITERATURE

Serotonin is a chemical substance found throughout nature. The actions of serotonin were originally discovered through observations of blood sera. Hillegaart (16) states that Stevens and Lee in the year 1884 and Brodie in the year 1900 observed that after blood clotted the remaining serum increased vascular tone, an observation that led directly to the name of the substance as serotonin. As Hillegaart points out (16), it was not until 1948, however, that Rapport et al. identified the specific chemical responsible for the initially observed vasoconstrictor qualities and assigned the name of 5-hydroxytryptamine (5-HT or serotonin) to that substance (Figure 1).

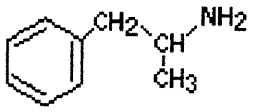
Later discoveries demonstrated that 5-HT is found in a variety of locations in the body. Platelets, mast cells and the enterochromaffin cells of the intestinal mucosa all were shown to contain significant amounts of 5-HT (7). Platelets, which cannot produce 5-HT, actively take-up and appear to store 5-HT for release when the platelets are damaged. Serotonin released from damaged platelets has been implicated in platelet activation, in vascular smooth muscle cell and connective tissue cell proliferation, and in thrombus formation (43). In the intestines, serotonin is associated with the regulation of peristaltic activity of the intestines (43). Serotonin was also found in the nervous system of many organisms (16). Hillegaart points out that in 1953 Twarog and Page demonstrated the presence of 5-HT in the brain of mammals. As described in Hillegaart (16), in 1956 Bogdanski et al. and in 1957 Brodie and Shore independently suggested that serotonin is a neurotransmitter, based upon earlier evidence that 5-HT is not distributed uniformly throughout the brain.

The serotonin produced outside the central nervous system cannot cross the blood/brain barrier. Consequently, the 1-2% of the body's 5-HT that is found in the

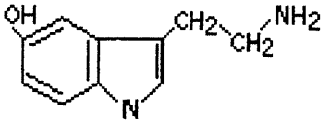
3,4-Methylenedioxy-methamphetamine



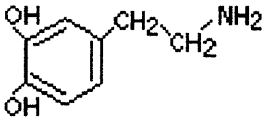
Amphetamine



Serotonin (5HT)



Dopamine



Norepinephrine

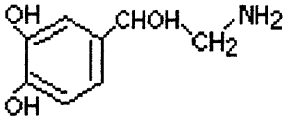


Figure 1. Molecular Structure of MDMA, amphetamine, serotonin, dopamine, and norepinephrine.

brain must be manufactured by serotonergic neurons within the central nervous system. The precursor to 5-HT, tryptophan, is primarily obtained via dietary sources. Tryptophan that circulates in the blood competes with other neutral amino acids for active transport into the brain across the blood/brain barrier (7, 43). Once across the barrier, tryptophan is taken up by nerve cells which can then convert the amino acid to serotonin. Conversion begins when tryptophan is hydroxylated at the 5 position by the enzyme tryptophan hydroxylase to produce 5-hydroxytryptophan. An amino acid decarboxylase then removes the carboxyl group from 5-hydroxytryptophan, to produce 5-hydroxytryptamine (5-HT). Any excess serotonin is converted to 5-hydroxy indole acetic acid (5-HIAA) by monoamine oxidase and aldehyde dehydrogenase. 5-HIAA can then be transported out of the brain (7) and excreted by the kidneys.

Elucidation of the neurotopography of serotonergic pathways was made possible by the fluorescent histochemical technique developed by Falck and Hillarp (7, 16) and refined through a combination of more recent techniques involving orthograde and retrograde tracking of immunohistochemically labeled 5-HT.

Research has identified distinct groups of serotonergic neurons along or in close proximity to the midline or raphe areas of the pons and upper brain stem (7, 16, 43). The original nine serotonergic nuclei (B1-B9) described by Dahlstrom and Fuxe in 1964 has been expanded to include 5-HT containing cells in the area postrema, the caudal locus ceruleus, and the region in and around the interpeduncular nucleus (7). Lesioning and orthograde/ retrograde tracing studies have shown that the more caudal cell groups (B1-B3) project to the medulla and spinal cord, the more rostral cell groups (B7-B9) project to the telencephalon and the diencephalon, and the intermediate 5-HT cell groups project in both ascending and descending directions (7, 16).

The structure and function of serotonergic cells are addressed by Whitaker-Azmitia (43, see also 7, 16). According to Whitaker-Azmitia, the serotonergic cells of

rats begin to express their neurotransmitter phenotype as early as embryonic day 12. Whitaker-Azmitia also describes the 5-HT receptor types associated with serotonergic cells. The receptor types include three 5-HT_1 receptors (5-$HT_{1A, 1B, 1D}$), three 5-HT_2-type receptors (5-$HT_{1C, 2A, 2B}$), and one 5-HT_3 receptor. The 5-HT_1 receptors work through second messengers that inhibit adenylate cyclase. Two of the 5-HT_2 (5-$HT_{1C, 2B}$) receptors have been shown to exert their effects by mediation of the second messengers inositol triphosphate and diacylglycerol. The mode of action of 5-HT_{2A} and the single 5-HT_3 receptor types is unknown (43).

The large number of 5-HT receptors would seem to suggest a highly complex role for serotonin in the body. In fact, serotonin has been associated with a wide range of behaviors and psychological conditions (16, 43). For example, serotonergic systems have been implicated in sexual and exploratory behavior as well as thermoregulation in laboratory rats (16). Whitaker-Azmitia (43) states that 5-HT also plays a role in the sleep-wake cycle, seizures, appetite, aggression, release of certain hormones, and circadian rhythms. These observations coincide with the assertions made by Cooper, Bloom, and Roth (7) that the serotonergic system mediates general homeostatic functions. Evidence also suggests that conditions such as migraine, eating disorders, affective illness, hyper-aggressive states, schizophrenia, depressive illness and suicidal behavior are accompanied by impaired or altered functions of serotonergic systems (15, 41, 42, 43).

Amphetamines and amphetamine-like drugs (Figure 1) affect specific neuropathways of the mammalian brain. Cooper, Bloom, and Roth (7) discuss the effects of amphetamine on catecholamine pathways in the brain. At the cellular level, the hypothesized modes of action for amphetamine fall into four categories (7, 18). Amphetamine may act as 1) an agonist at norepinepherine (NE) receptors, 2) an inhibitor of catecholamine reuptake, 3) a monoamine oxidase inhibitor, or 4) a promoter of NE and

dopamine (DA) release by a displacement process. Kandel and Schwartz (18) suggest that the most likely of the postulated mechanisms for amphetamine action includes promoting the release and blocking the reuptake of NE and/or DA. Evidence indicates that prolonged administration of amphetamine leads to an inactivity of catecholamine neurons, presumably due to depletion of the transmitter substance. Whatever the mechanism may be, the net result of amphetamine action appears to be an initial increase in activity, followed by a long-term decrease in activity at affected synapses (7, 18).

3,4-Methylenedioxymethamphetamine (MDMA, "Ecstasy," empirical formula $C_{11}H_{15}NO_2$ - Figure 1) is a ring-substituted phenylisopropylamine similar in nature to amphetamines and hallucinogens. Following the original patent of MDMA, issued in 1914 by the German company E. Merck (37), little attention was given to the drug until the last decade. The stimulant and psycho- mimetic effects of MDMA have made it a popular recreational drug as well as a controversial adjunct to psychotherapy (6, 12). The appeal of MDMA to some therapists rests on reports of lowered anxiety levels and reduced defensiveness in patients treated with MDMA (12).

Studies have demonstrated drawbacks to casual use and treatment with MDMA (6, 11, 24, 34, 35). Recreational use of the drug by humans has been directly linked to one death and secondarily associated with several other fatalities (6, 11). Series and coworkers (36) recently reported two cases, one a 24-year-old male and the other a 23-year-old female, that suggest recreational use of "Ecstasy" produces significant psychiatric disorders. Self-administration studies on MDMA (4, 37) suggest a high potential for abuse of the drug by humans and long-term use has been correlated with severe neurotoxicity (3, 6, 8, 24). In fact, recognition of the adverse effects of MDMA led to it being classified as a schedule 1 drug.

A relatively recent study by McCann et al. (25) demonstrates the dangers of MDMA use by humans. McCann et al. performed a controlled study in which humans

were administered MDMA and measurements of biological and behavioral factors related to serotonergic function in the central nervous system were made. The measurements of 5-hydroxy-indoleacetic acid levels in the CNS, as well as of the behavioral factors of impulsivity and indirect hostility, present substantial and significant evidence to support the assertion that MDMA is toxic to human beings.

The specific mechanisms by which MDMA produces its effects are unclear. However, research using animal paradigms has offered insight into the neurochemical effects and neurotoxicity of MDMA. For example, evidence gathered using animal models indicates that it is unlikely that MDMA is transported into nerve terminals via carrier-mediated processes (26, 31, 33). Because MDMA does not appear to be taken directly into cells, other modes of action have been postulated. Receptor-mediated actions offer a possible alternative mode of action for MDMA. The affinity of MDMA and its optical isomers for various receptors and receptor subtypes has been examined by several researchers. The R(-)-MDMA isomer has been shown to have a higher affinity for examined receptors than the S(+)-MDMA isomer or racemic MDMA (20). In general, MDMA shows the greatest affinity (K_i 0.6 +/- 0.05 *u*M) for serotonergic uptake receptors; it shows less affinity for 5-$HT_{2A, 1A, 1D,}$ and $_{2B}$ receptors and the adrenergic a_2, b, and a_1; it shows even less affinity for the dopaminergic receptors D_2 and D_1 (4, 20).

Toxic metabolites may also be involved in the neurotoxicity produced by MDMA. There are several arguments for and against the role of toxic metabolites of one form or another in MDMA induced neurotoxicity. McKenna and Peroutka (26) suggest that because 5-HT uptake inhibitors block MDMA-induced neurotoxicity (29, 30) and only systemic administration of MDMA (as distinct from intracerebral administration) produces neurotoxicity, toxic metabolites of MDMA appear to be involved. Yeh and Hsu (44) offer evidence in opposition to the hypothesis that toxic metabolites are involved;

these researchers demonstrated that the neurotoxic effects of MDMA are much more profound than the toxic effects of the metabolites of MDMA. McKenna and Peroutka (26) offer another suggestion; they propose that MDMA-induced neurotoxicity might be due to toxic metabolites produced by degradation of dopamine (DA) rather than metabolites of MDMA. They argue that MDMA may cause inhibition of the enzyme monoamine oxidase and thus allow increased levels of DA around 5-HT neurons. The DA is then presumably taken up and metabolized to cytotoxic metabolites via oxidative processes. The suggested role of DA involvement in MDMA induced serotonergic toxicity stems from research done by Stone et al. in 1988 (38). Stone and coworkers demonstrated that by depleting the DA content in the central nervous system by administering a-methyl-*p*-tyrosine to block DA synthesis, they could significantly reduce the short- and long-term neurotoxic effects of MDMA.

McKenna and Peroutka (26, see also 32) divide the neurochemical effects of MDMA into acute (< 24 hrs.) and long-term (> 24 hrs.) stages. The acute effects include decreased tryptophan hydroxylase (TPH) activity as well as significantly decreased brain concentrations of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA). The low concentration of brain hydroxyindoles, thought to be related to massive neuronal release of 5-HT, return to normal levels within 24 hours. After the first 24 hours, MDMA causes several long-term neurochemical effects. For example, following the initial return to normal levels, 5-HT and 5-HIAA concentrations follow a slow and regular decrease. Long-term effects of MDMA are further characterized by a persistent decrease in TPH activity and a decrease in 5-HT terminal concentrations (26). In summary, the long-term neurochemical effects are indicative of severe serotonergic depletion. Distinct from amphetamine, which affects catecholamine pathways, the severe damage incurred by systemic MDMA administration has been shown to include degeneration of fine serotonergic axons in cortical, hippocampal, and striatal brain

regions (2). As extensive as the axonal damage is, the pattern of damage suggests that the toxic effects of MDMA do not involve cell death - this fact raises the question of whether or not it would be possible to generate new fibers to replace those previously damaged.

Repair of neuronal damage in the central and peripheral nervous systems involves enhancing neuroplasticity mechanisms inherent in the systems and the cells of the systems. Complex biological molecules called gangliosides demonstrate the ability to trigger neurotrophic and neuroprotective effects associated with general neuroplasticity. Gangliosides are glycosphingolipids with a short carbohydrate chain attached to a lipophilic (hydrophobic) tail consisting of an amino alcohol sphingosine and a fatty acid. Research on the effects of the neutral lipid monosialoganglioside GM_1 on neural fibers of the central nervous system has demonstrated that it offers protection against physical and chemical damage (22, 23, 27).

Evidence in favor of the efficacy of treatment with gangliosides and, specifically, GM_1 can be found in studies on the effects of GM_1 in facilitation of recovery from various nervous system injuries. The neuroplasticity enhancing affects of GM_1 have been examined in cases ranging from chemical (9, 10, 13, 19, 21) and physical lesions (5) to injury resulting from ischemic stroke (1, 17).

Endogenous GM_1 is a normal component of cell membranes but is especially prevalent in the CNS, where it is associated with a multitude of physiological activities involving these membranes. GM_1 is thought to exert its affects once incorporated into the cell membrane. In fact, exogenously administered GM_1 has been shown to be efficiently incorporated into isolated neuronal membranes *in vitro* (27). Although the specific mechanisms responsible for the observed neurotrophic and neuroprotective effects of GM_1 are not known, research has shown several general modes of action. For example, GM_1 inhibits neuronal fiber degeneration and accelerates functional

synaptogenesis in lesioned tissue (22, 27). Studies (19, 23) demonstrate that GM_1 inhibits neuronal damage mediated by excitatory amino acids. Specifically, Manev et al. (23) discuss a mechanism by which GM_1 protects neurons from chemical (glutamate)-induced necrosis. Manev and coworkers (23) postulate that exogenously administered gangliosides such as GM_1 are incorporated into the cell membrane where they inhibit protein kinase C destablization of Ca^{2+} homeostasis in the cell. Inhibition of protein kinase C appears to be due to a direct blockade of the cascade of which the kinase is a part. Since Ca^{2+} levels are closely related to necrosis caused by excessive stimulation by excitatory amino acids (ie. glutamate), prevention of the destablization prevents the glutamate-induced toxicity. Other possible mechanisms by which GM_1 facilitates neuroplasticity can be found in research that demonstrates that treatment with GM_1 increases ATPase activity, enhances protein phosphorylation, promotes neurite outgrowth and prolongs *in vitro* survival of isolated neurons (27).

The demonstrated protective and neurotrophic abilities of GM_1 implicate it as a possible treatment for a wide range of physical lesions and as a possible antagonist to the effects of drugs shown to have neurotoxic tendencies.

The purpose of this study was to explore the hypothesis that the rate of recovery from MDMA-induced serotonergic neurotoxicity could be enhanced via collateral action of the ganglioside GM_1 by its reported neurotrophic properties.

MATERIALS AND METHODS

Experimental Design

This study was designed to examine the efficacy of systemically administered GM_1 to attenuate the neurotoxic effects of MDMA on the rat brain. Male Sprague-Dawley rats (250g to 350g) were used in a 4 X 2 block style experimental design. The treatment groups included: a) saline solution (0.9%), b) MDMA, c) GM_1 and d) MDMA + GM_1. Animals were sacrificed either 7 or 14 days following the beginning of the drug treatment. All drugs were administered by intraperitoneal injections in volumes of 0.2-0.3 ml. MDMA obtained from Research Biochemicals International (RBI, Natick, MA) was administered at a dose of 20 mg/kg once a day for four days. GM_1 (kindly provided by FIDIA Pharmaceuticals, Italy) was administered as a single 40 mg/kg dose. When given concomitant with MDMA, GM_1 was administered on the first day of the MDMA treatment. Five animals per treatment group per sacrifice day were used for a total of 40 animals. Table 1 depicts the research design.

Table 1. The various parameters of the experiment including treatment, sacrifice day, and number of animals used for each phase of the experiment

	Saline		MDMA		GM_1		MDMA +GM_1	
Sacrifice day	7	14	7	14	7	14	7	14
Behavioral/ Biochemical	n=5	n=5	n=5	n=5	n=5	n=5	n=5	n=5

Behavioral Activity

The behavioral activity study was performed in the familiar surroundings of the animal care facility. A 12-hour light/dark cycle was maintained and ambient room temperature was held at a constant 74° F (± 2°). Animals were placed in clear plastic cages with free access to food and water between 12:00 PM and 1:00 PM at day 5 or day 12 following the first day of injections. Each cage was placed on a Stoelting electromagnetic activity monitor that recorded the movements of the animals. The activity of the animals was measured in the form of the number of movements each animal made in 6-minute epochs throughout the 24-hour period on the monitor. The activity per 6-minute epoch was summed to give the total activity for each hour during the 24-hour period. The average activity, per hour, was then determined for each treatment group. At the conclusion of the activity monitoring period the animals were returned to their home cages until they were used for the biochemical analysis.

Data collected was analyzed using ANOVA and Tukey statistical tests when appropriate.

Biochemical Analysis

At day 7 or day 14 following the first day of injections, the rats from the behavioral activity analysis (24 hours after the behavioral activity study) were anesthetized with sodium pentobarbital (30 mg/kg) and sacrificed by cervical dislocation. The brains were then removed and the frontal cortex, lateral cortex (containing the hippocampus), striatum/midbrain, cerebellum, and brainstem regions were separated by dissection (see Figure 2). Throughout the dissection, the brains were kept on ice and following dissection each region was weighed and stored at -70° C until assayed.

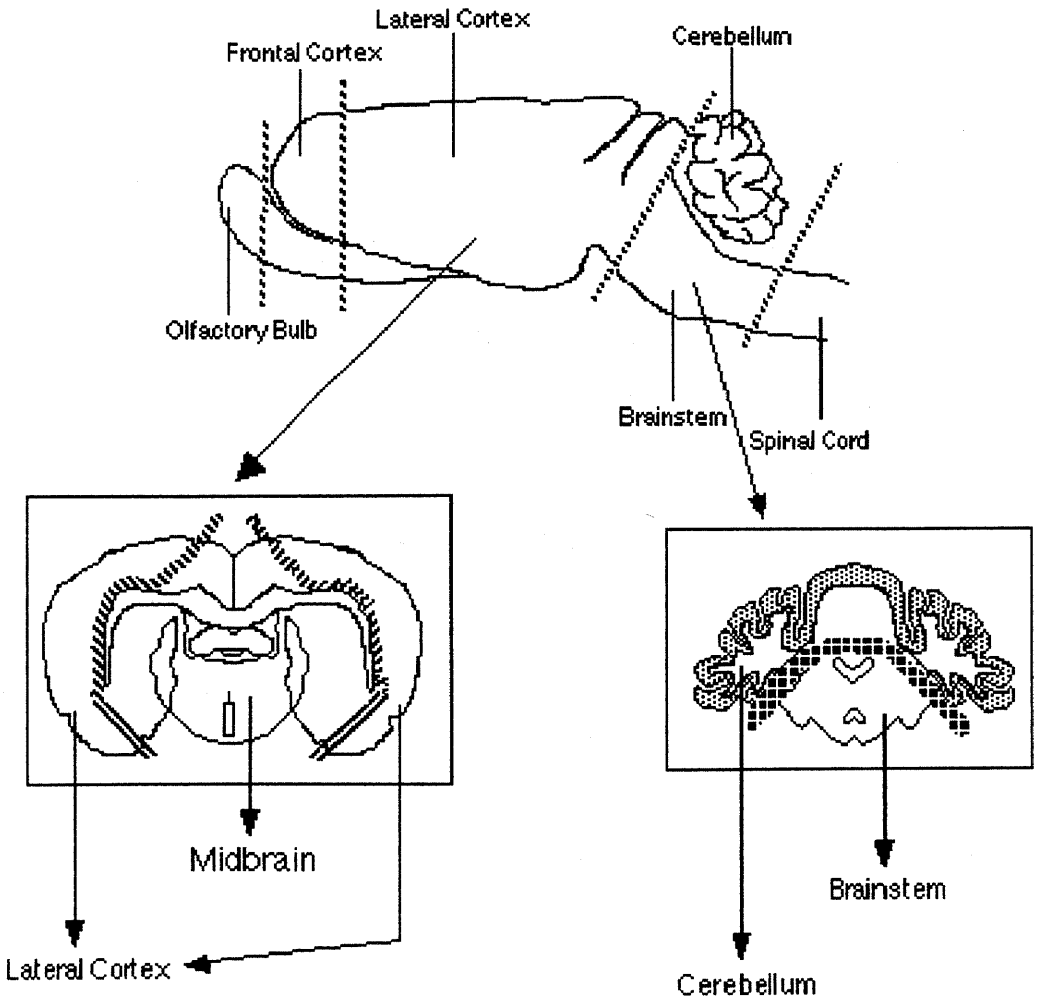


Figure 2. Diagram indicating cuts to obtain the brain regions to be analyzed. Patterned lines indicate cuts made. Regions include brainstem, lateral hemispheres, cerebellum, frontal cortex, and midbrain.

Preparation and assay of the samples were according to methods described by Saller and Salama (28, see also 39, 40). At the time of the assay, samples were placed in sonication cuvettes containing 38 volumes (volume = vol = the mass, in grams, of the tissue sample converted to a volume, in millileters) homogenization buffer and 2 vol of an internal standard. The homogenization buffer contained 7 vol 0.1 M monobasic sodium phosphate (pH 4.0) with 1 mM disodium EDTA and 1 mM sodium octanesulfonic acid and 3 vol acetonitrile. The internal standard, isoproternol, was added to 2 vol of the buffer, which was then added to the sample. Homogenization of the sample was accomplished with a W-185 sonicator (Heat Systems-Ultrasonics Inc., Plainview, NY). Following sonication, the samples were placed in 15 ml glass centrifuge tubes and centrifuged at 10,000 rpm for 10 minutes. A portion of the supernatant was removed from each sample and frozen at -70^{o} C until the time of the assay.

Before the HPLC assay the samples were thawed and re-centrifuged. The samples were then analyzed for serotonin (5-HT), 5-hydroxyindolacetic acid (5-HIAA), norepinepherine (NE), dopamine (DA), and dioxyphenylacetic acid (DOPAC) using high performance liquid chromatography techniques that employed an electrochemical detection system. A small portion (20 µl) of each sample was manually injected using a Beckman 210A injector (Beckman Instruments, Inc. Berkley, CA). Following injection, the sample passed through a 0.46 X 4.5 cm guard column and then through an Altex 5-µm, 0.46 X 25 cm C_{18} Ultrasphere reverse-phase column (Beckman Instruments, Inc.). Electrochemical detection was was accomplished as the column effluent passed through a Bio-Rad Model 1340 electrochemical detector (Bio-Rad Laboratories, Hercules, CA) with a 0.65 V electrical potential applied to the working electrode. The resultant peak information was integrated by a Hewlett-Packard Model 3392a integrator (Hewlett-Packard Co., Avondale, PA). Dr. Dean Hoganson at Drake University supervised the biochemical analysis procedure.

Collected data was analyzed using ANOVA and Tukey statistical tests when appropriate.

This research protocol was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Osteopathic Medicine and Health Science. The methods were found to meet the revised policy on the care of animals in neuroscience research (Society for Neuroscience).

RESULTS

Behavioral Activity

The average activity (measured as movements) for the animals in each treatment group for the full 24-hour period is depicted graphically in Figures 3b and 4b. As can be seen, at both day 5 and day 12 following the beginning of the treatments, the animals showed an initially high level of activity that decreased to a lower less variable level. The initial high level of acitivity corresponds to the normal exploratory behavior of the animals, while the decrease and levelling off reflects the acclimation of the animals to their surroundings. Furthermore, as Figures 3b and 4b suggest the greatest variability among treatment groups can be found during the exploratory phase of the animals.

An ANOVA and multiple comparisons (Tukey) analysis of the data collected throughout the behavioral study demonstrated that the majority of the significant differences between treatment groups fell within the first 5 hours (13:00 to 17:00) of activity for both day 5 and day 12. As a result of the statistical analysis for the behavioral data collected for the full 24 hour period, attention was focused on analyzing the statistically significant differences found within the animals' exploratory phase (Tables 2-5 and Figures 3a-4a).

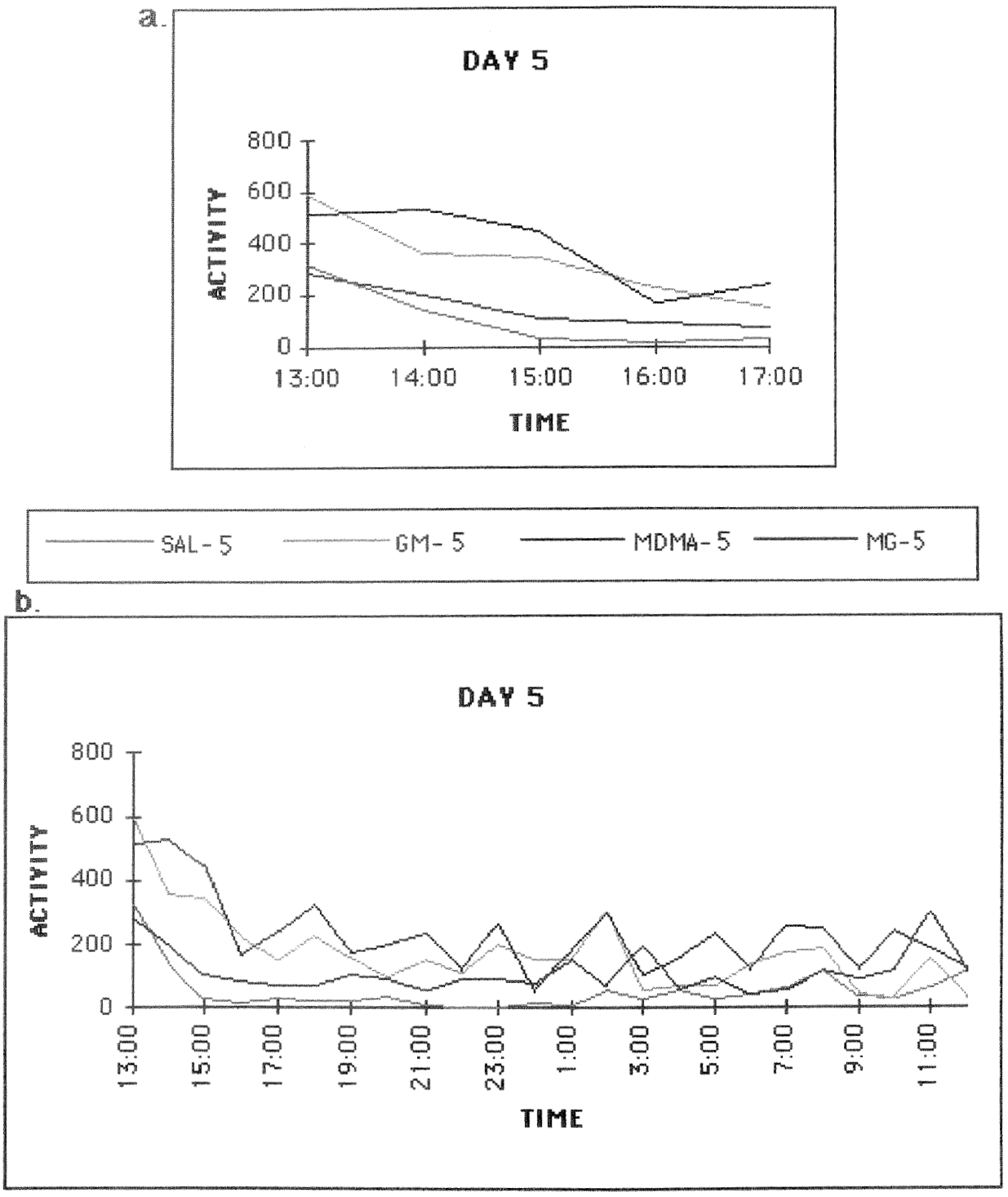


Figure 3. Average summed behavioral activity at day (in movements) for each treatment group for the first five hours (a.) and the full 24 hour period (b.).

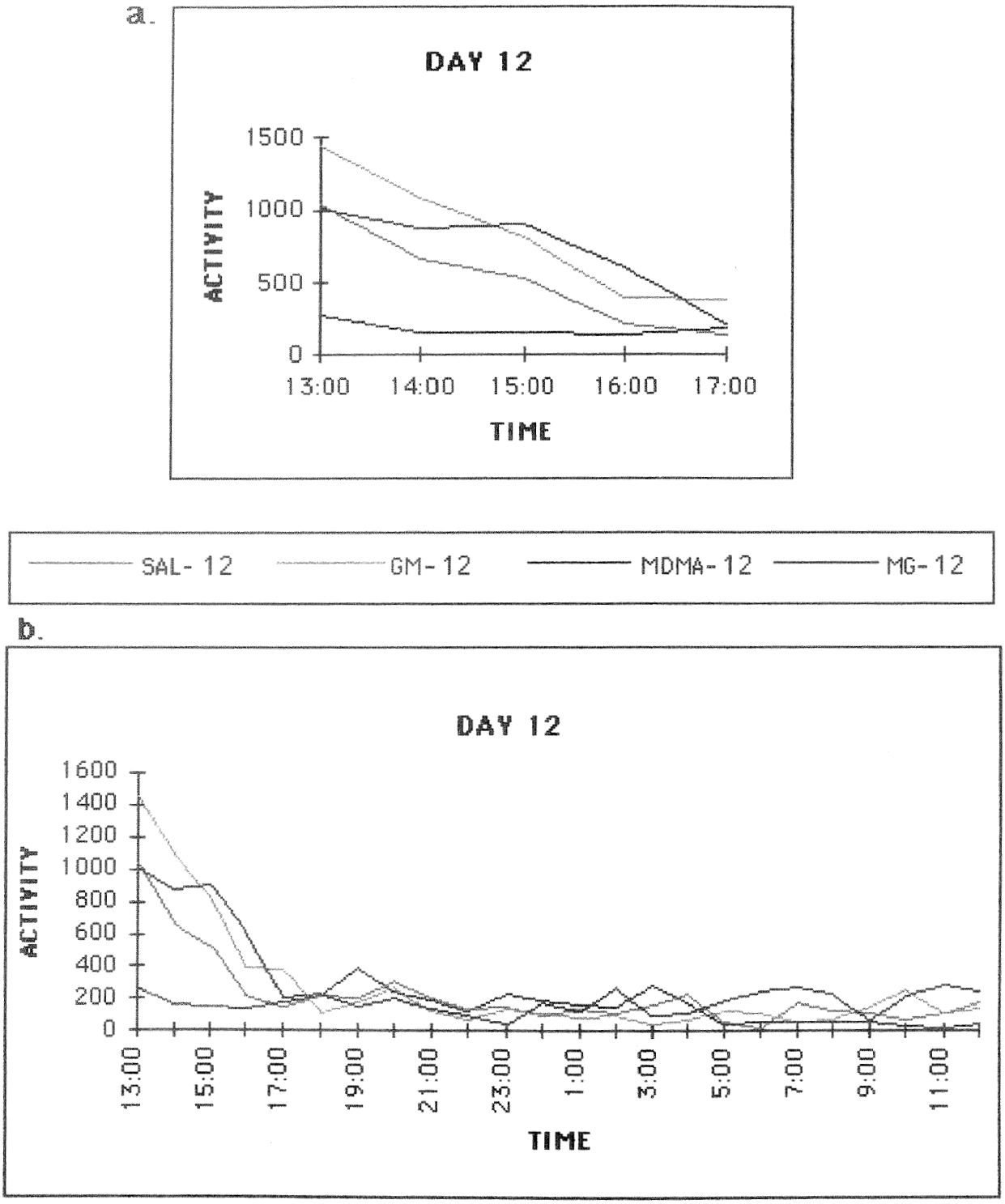


Figure 4. Average summed behavioral activity at day 12 (in movements) for each treatment group for the first five hours (a.) and the full 24 hour period (b.).

At day 5, the first five hours of activity (Tables 2-3 and Figure 3a) of the animals fall within two distinct categories based on statistical similarities and differences. The first category includes the activity of the GM_1 and MDMA groups. The summed activity for the first hour of each of these groups falls between 500 and 600 movements and drops to between 200 and 300 movements by the fifth hour. Compared to one another, the GM_1 and MDMA treated animals do not show significantly different levels of activity within the first five-hour period. However, the activity of the GM_1 and MDMA treated animals is significantly greater than the level of activity expressed by the controls and the MDMA + GM_1 treated animals. The control and collateral treatment groups (MDMA + GM_1) make up the second category of activity, in that they are not significantly different from each other but are significantly lower than the GM_1 and MDMA groups. The activity level of those animals in the second category begins at approximately 300 movements in the first hour and drops to between 50 and 100 movements by the fifth hour.

In the first five hours at day 12 (Table 4-5 and Figure 4a), the activity of the control animals, those animals treated with GM_1, and those animals that received collateral treatment were not significantly different from each other, however, they were all significantly greater than the level of activity of the MDMA treated animals. In the first hour, the average activity was approximately 1490 movements for the animals treated with GM_1, 1000 movements for the controls and collaterally treated animals, and 300 movements for the MDMA treated animals. By the fifth hour, the level of activity of each group had dropped slightly.

Despite the 12-hour light-dark cycle, no noticable change in activity level was observed corresponding to the light and dark periods.

Table 2. Behavioral activity at day 5 for the first 5 hours

Treatment	Time				
	13:00	14:00	15:00	16:00	17:00
Control	321 ± 276	142 ± 253	34 ± 47	16 ± 16	30 ± 51
GM_1	589 ± 339	357 ± 277	347 ± 315	225 ± 241	151 ± 107
MDMA	514 ± 150	532 ± 225	446 ± 219	167 ± 172	240 ± 252
MDMA + GM_1	427 ± 255	200 ± 181	104 ± 39	87 ± 48	70 ± 15

Data are mean ± SD. Time indicated is from a 24 hour scale. Control = the saline controls, GM1 = group given the GM1 alone treatment, MDMA = group given the MDMA alone treatment and MDMA + GM1 = the collateral treatment group.

Table 3. ANOVA of behavioral activity in the first five hours at day 5

	SS	df	MS	F	P-value
Time	1338289.5	4	334572.4	8.7	7.20E-06
Treatment	1346709.5	3	448903.2	11.6	2.10E-06
Interaction	267582.6	12	22298.6	0.6	0.9
Within	3083863.2	80	38548.3		
Total	6036444.8	99			

Time (hours 13:00- 15:00 on a 24 hour scale) by treatment (control, GM_1, MDMA, and MDMA + GM_1). SS = sum of squares, df = degrees of freedom, MS = mean of squares, F = F-value and P-value = P-value. Tukey's multi-comparisons test showed that there were significant differences between the controls and the GM_1 treated groups, the controls and the MDMA treatment group, the GM_1 group and the MDMA + GM_1 group, as well as the MDMA group and the MDMA + GM_1.

Table 4. Behavioral activity at day 12 for the first 5 hours

Treatment	Time				
	13:00	14:00	15:00	16:00	17:00
Control	1040 ± 577	663 ± 462	532 ± 512	220 ± 199	146 ± 63
GM_1	1452 ± 698	1084 ± 483	823 ± 338	395 ± 485	378 ± 243
MDMA	272 ± 331	163 ± 68	150 ± 53	138 ± 66	179 ± 113
MDMA + GM_1	1004 ± 342	874 ± 469	903 ± 714	601 ± 196	213 ± 136

Data are means ± SD. Time indicated is from a 24 hour scale. Control = the saline controls, GM1 = group given the GM1 alone treatment, MDMA = group given the MDMA alone treatment and MDMA + GM1 = the collateral treatment group.

Table 5. ANOVA of behavioral activity in the first 5 hours at day 12

	SS	df	MS	F	P-value
Time	1338289.5	4	334572.4	8.7	7.20E-06
Treatment	1346709.5	3	448903.2	11.6	2.10E-06
Interaction	267582.6	12	22298.6	0.6	0.9
Within	3083863.2	80	38548.3		
Total	6036444.8	99			

Time (hours 13:00-15:00 on a 24 hour scale) by treatment (control, GM_1, MDMA, and MDMA + GM1).SS = sum of squares, df = degrees of freedom, MS = mean of squares, F = F-value and P-value = P-value. Tukey's multi-comparisons test showed that there were significant differences between the controls and each of the other treatment groups (MDMA, GM_1, and MDMA + GM_1), between the GM_1 group and the MDMA group, and between the MDMA group and the MDMA + GM_1 group.

Biochemical Analysis

Brain tissue from the different regions (brainstem = BS, lateral hemispheres = LH, cerebellum = CB, frontal cortex = FC, and midbrain = MB; see Figure 2) was assayed for serotonin (5-HT), dopamine (DA), norepinephrine (NE), 5-hydroxyindoleacetic acid (5-HIAA), and dioxyphenylacetic acid (DOPAC). The results of the assays are given in Tables 6-10 and graphically depicted in Figures 5-9.

Concentrations of the neurotransmitters and related metabolites within the control groups varied by region as would be expected. 5-HT concentration was the greatest in the midbrain region corresponding to the raphe. NE concentrations of 400-500 ug/gm were observed in the brainstem and midbrain regions which contain the locus ceruleus. The greatest concentrations of DA were found in the lateral hemispheres and the midbrain, slices which were likely to contain part or all of the striatum and substantia nigra. 5-HIAA was found in relatively high concentrations in all of the tissues except the cerebellum and observed to be regionally distributed in a manner similar to its parent transmitter, 5-HT. The concentration of DOPAC, on the other hand, was minimal to non-existent in all of the tissues examined.

In every tissue region analyzed, the MDMA treatment significantly reduced the neurotransmitter (5-HT, NE, and DA) concentrations at both day 7 and day 14. For 5-HT and NE, the GM_1 treatment also significantly reduced the transmitter concentrations at day 7 and day 14. The collateral treatment group (MDMA+GM_1) showed an appreciable, albeit non-significant, attenuation of the MDMA-induced reduction of the transmitters 5-HT, DA, and NE. The most dramatic effects were observed for the DA (see Figure 6).

The concentration of the metabolite of 5-HT (5-HIAA) was reduced by MDMA and MDMA + GM_1 treatments. Interestingly, there was little or no effect with GM_1 treatment and no appreciable attenuation of MDMA effect by the collateral treatment with GM_1.

Sample	Day 7			Day 14		
Brainstem	[5-HT] ug/gm	Std. Dev.		[5-HT] ug/gm	Std. Dev.	
CONTROL	192.4	±154.7		352	±44.82	
GM1	55.6	±58.18		104.6	±97.59	X
MDMA	1	±2.24	X	9.8	±16.04	X
MDMA + GM1	23.2	±51.88	X	29	±41.69	X
Lateral hemispheres						
CONTROL	264	±56.97		297.5	±95.57	
GM1	112.4	±58.93	X	193	±32.78	
MDMA	36.6	±24.13	X	45.8	±78.59	X
MDMA + GM1	52.8	±75.3	X	91.6	±64.23	X
Cerebellum						
CONTROL	36	±16.55		59.25	±18.43	
GM1	15.6	±28.18		4	±8.94	X
MDMA	0	±0	X	0	±0	X
MDMA + GM1	0	±0	X	26.2	±16.98	X
Frontal Cortex						
CONTROL	128.6	±78.67		164.5	±97.19	
GM1	28.2	±35.07		56	±64.85	
MDMA	0.8	±1.79	X	8.6	±19.23	X
MDMA + GM1	4.4	±9.84	X	1.4	±1.95	X
Midbrain						
CONTROL	335.4	±145.9		550.25	±182.7	
GM1	297.8	±110.2		317.4	±176.5	
MDMA	11.4	±25.5	X	127.2	±42.6	X
MDMA + GM1	71.4	±60.5	X	127.2	±97.2	X

Table 6: Serotonin (5-HT) concentration (ug/gm of tissue, wet weight) at day 7 and 14 for each of the treatment groups by brain region analyzed; X = significantly different from control ($p < 0.05$).

Sample	Day 7			Day 14	
Brainstem	[DA] ug/gm	Std. Dev.		[DA] ug/gm	Std. Dev.
CONTROL	57.8	±29.98		30	±12.14
GM1	0	±0	X	3.4	±7.6
MDMA	0	±0	X	5	±11.18
MDMA + GM1	6	±8.49	X	16.4	±27.32
Lateral hemispheres					
CONTROL	404	±213		480.75	±267.1
GM1	283.6	±260.6		256.8	±180.4
MDMA	136.8	±119.6		65.8	±68.5
MDMA + GM1	321	±429.3		427.8	±324.3
Cerebellum					
CONTROL	4.2	±9.39		11.25	±14.73
GM1	13.4	±14.03		0	±0
MDMA	12.2	±12.36		4.8	±10.73
MDMA + GM1	4.4	±9.84		33	±36.11
Frontal Cortex					
CONTROL	207	±276.4		269.75	±253.1
GM1	28.8	±23.2		26.8	±21.1
MDMA	10.8	±17.4		16.4	±28.6
MDMA + GM1	18.2	±13		11.6	±10.9
Midbrain					
CONTROL	260.2	±63.5		617	±443.6
GM1	323	±248.9		397.8	±243.8
MDMA	61.2	±50.8		176.4	±154
MDMA + GM1	141	±110.3		215.4	±214.9

Table 7: Dopamine (DA) concentration (ug/gm of tissue, wet weight) at day 7 and 14 for each of the treatment groups by brain region analyzed; X = significantly different from control ($p < 0.05$)

Sample	Day 7			Day 14		
Brainstem	[NE] ug/gm	Std. Dev.		[NE] ug/gm	Std. Dev.	
CONTROL	427.4	±184.63		498.75	±49.28	
GM1	51	±60.67	X	99.4	±84.53	X
MDMA	2.2	±4.92	X	0	±0	X
MDMA + GM1	38	±38.24	X	35.6	±56.01	X
Lateral hemispheres						
CONTROL	164.2	±36.97		223.25	±30.41	
GM1	50.4	±20.4	X	74.2	±14.1	X
MDMA	32	±21.83	X	22.6	±16.79	X
MDMA + GM1	64.8	±63.61	X	85	±39.29	X
Cerebellum						
CONTROL	82.2	±28.88		105.75	±14.06	
GM1	34.8	±37.33		16.4	±29.94	X
MDMA	6.2	±13.86	X	17.8	±26.44	X
MDMA + GM1	22.2	±31.86	X	55.4	±34.46	
Frontal Cortex						
CONTROL	134.6	±84.98		116.75	±82.27	
GM1	27.2	±17.75	X	28.8	±32.95	
MDMA	6	±9.59	X	5.4	±12.07	X
MDMA + GM1	11.2	±15.74	X	8.6	±12.64	X
Midbrain						
CONTROL	448.8	±82.9		575.75	±163.07	
GM1	161.2	±14.1	X	167.2	±82.86	X
MDMA	36.2	±34.82	X	130.8	±48.58	X
MDMA + GM1	108.4	±85.44	X	136.6	±107.46	X

Table 8: Norepinephrine (NE) concentration (ug/gm of tissue, wet weight) at day 7 and 14 for each of the treatment groups by brain region analyzed; X = significantly different from control ($p < 0.05$)

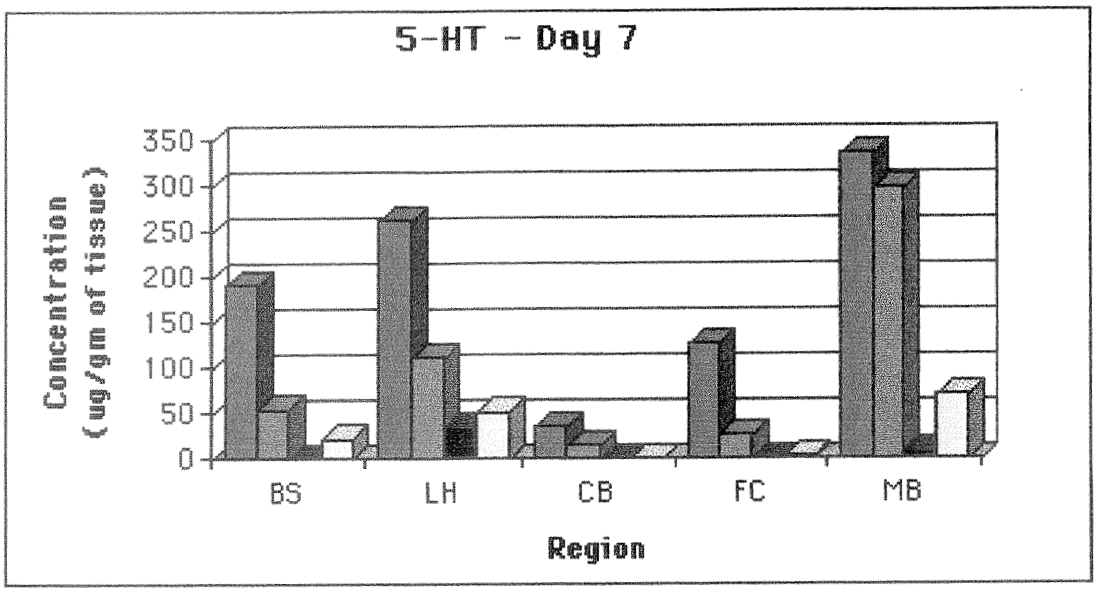
Sample Brainstem	Day 7 [5-HIAA] ug/gm	Std. Dev.		Day 14 [5-HIAA] ug/gm	Std. Dev.	
CONTROL	945.2	±249.7		751.5	±67.5	
GM1	1116	±370.5		1015.2	±229.3	
MDMA	627.4	±181.8	•	510.6	±74.3	•
MDMA + GM1	589.4	±170.1	•	724.2	±192.4	
Lateral hemispheres						
CONTROL	485	±96.2		668.4	±242	
GM1	486.6	±29		558.4	±76.5	
MDMA	373	±57.1		403.8	±257	
MDMA + GM1	254	±123.2		288.6	±61	X
Cerebellum						
CONTROL	151.2	±46.21		188.25	±22.85	
GM1	229.8	±30.13		234.6	±34.82	
MDMA	121	±32.23	•	143.4	±50.18	•
MDMA + GM1	131.4	±40.73	•	150.4	±41.78	•
Frontal Cortex						
CONTROL	671.8	±150.4		831.25	±326.2	
GM1	685.4	±312.3		620.6	±159.5	
MDMA	316.6	±138.6		425.4	±168.8	
MDMA + GM1	301.6	±134		521.2	±116	
Midbrain						
CONTROL	1009.6	±425.9		1045.25	±163.5	
GM1	1183.2	±206.6		1037.2	±163	
MDMA	722.4	±331.6		626.6	±134.6	
MDMA + GM1	743.6	±363.7		702.6	±308.3	

Table 9: 5-hydroxyindoleacetic acid (5-HIAA) concentration (ug/gm of tissue, wet weight) at day 7 and 14 for each of the treatment groups by brain region analyzed; X = significantly different from control ($p < 0.05$); • = significantly different from GM_1 treatment group.

Sample	Day 7			Day 14	
Brainstem	[DOPAC] ug/gm	Std. Dev.		[DOPAC] ug/gm	Std. Dev.
CONTROL	0	±0		22.5	±45
GM1	53	±43.21		48.4	±29.13
MDMA	12	±26.83		24.6	±26.2
MDMA + GM1	61.4	±70.44		52.4	±20.23
Lateral hemispheres					
CONTROL	0	±0		0	±0
GM1	299.8	±177.1		236	±114.2
MDMA	117.2	±57.4		186.6	±274.7
MDMA + GM1	243	±222.4		209	±69.6
Cerebellum					
CONTROL	3.2	±7.16		0	±0
GM1	17	±26.04		0	±0
MDMA	28.8	±64.4		6.8	±15.21
MDMA + GM1	40.8	±42.52		54.8	±69.97
Frontal Cortex					
CONTROL	68	±117.8		0	±0
GM1	250.2	±501.6		205.2	±195.2
MDMA	72.8	±162.8		33.2	±50.5
MDMA + GM1	145	±160.8		351.6	±552.1
Midbrain					
CONTROL	7.8	±17.4		34.5	±39.9
GM1	472.6	±243.2		658.6	±118.6
MDMA	261	±416.8		260.8	±142.3
MDMA + GM1	916.6	±549.4	X	503.6	±300.4

Table 10: Dioxyphenylacetic acid (DOPAC) concentration (ug/gm of tissue, wet weight) at day 7 and 14 for each of the treatment groups by brain region analyzed; X = significantly different from control ($p < 0.05$)

A.)



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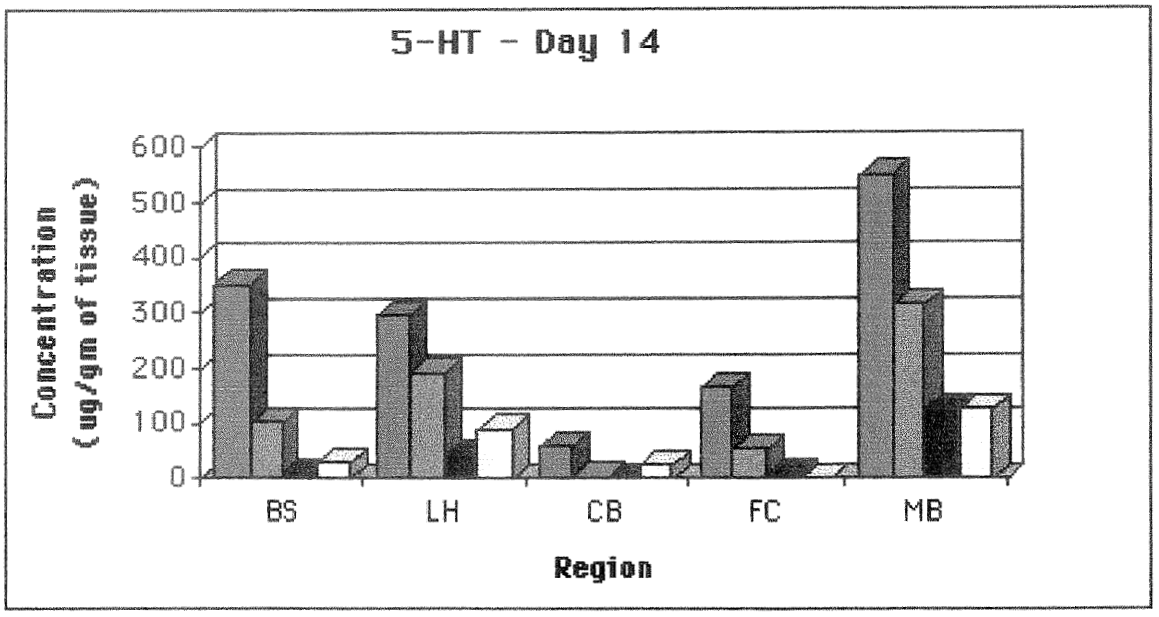
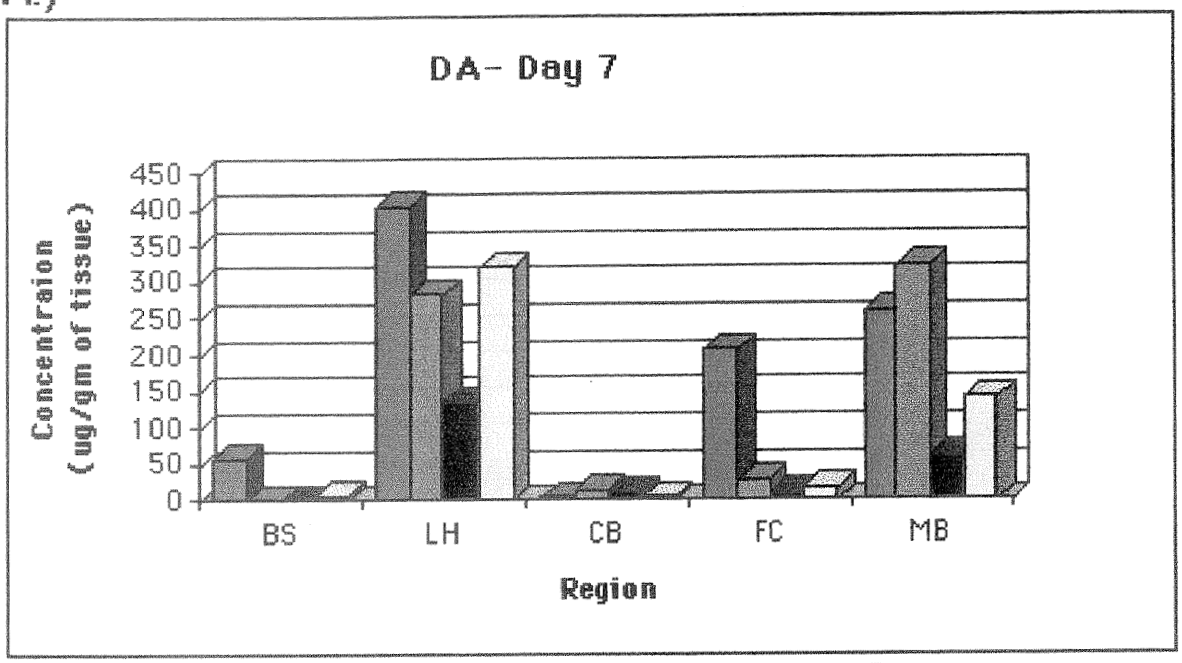


Figure 5. Average concentration of 5-HT for each of the brain regions analyzed. BS= Brainstem, LH=Lateral Hemispheres CB=Cerebellum, FC=Frontal Cortex, and MB=Midbrain. A.) Animals sacrificed 7 days following the beginning of injections. B.) Animals sacrificed 14 days following the beginning of injections.

A.)



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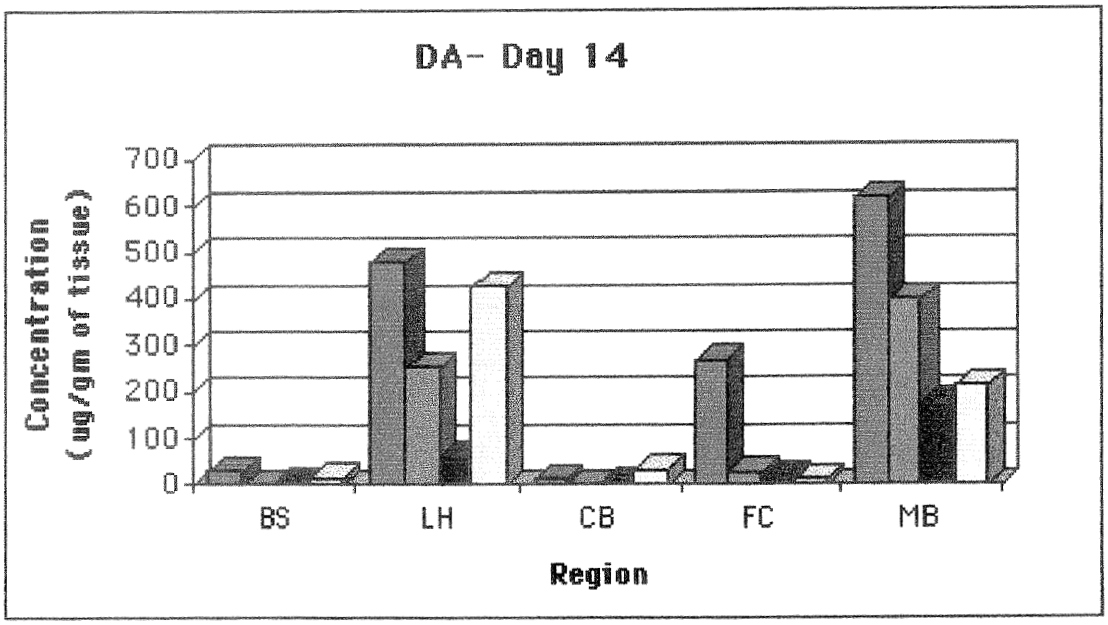
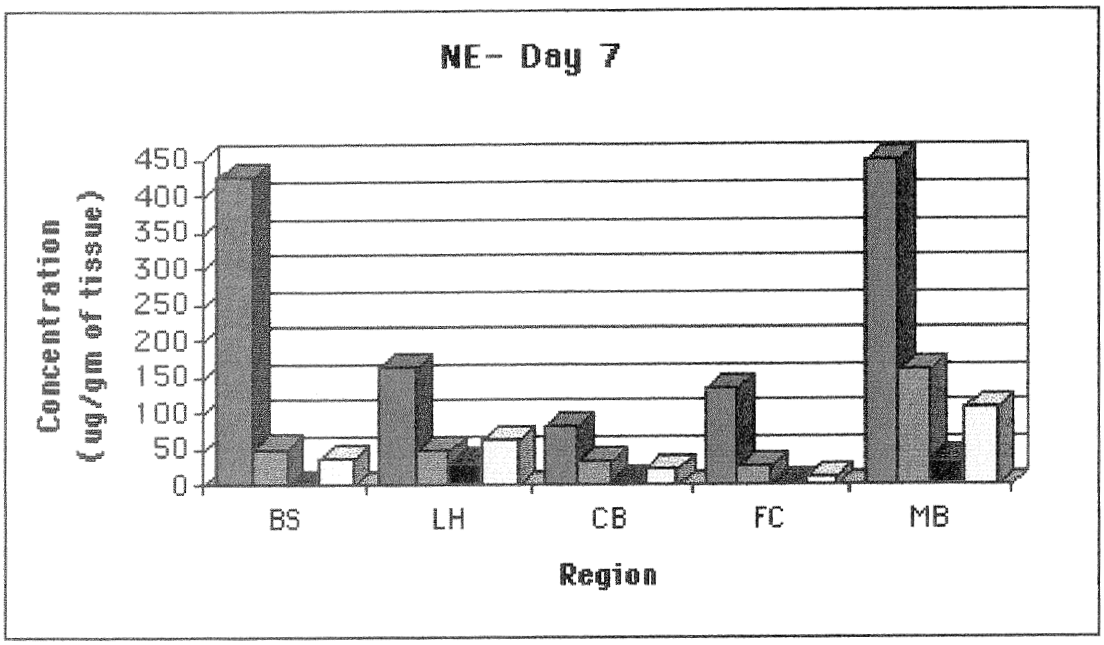


Figure 6. Average concentration of dopamine (DA) for each of the brain regions analyzed. BS= Brainstem, LH=Lateral Hemispheres CB=Cerebellum, FC=Frontal Cortex, and MB=Midbrain. A.) Animals sacrificed 7 days following the beginning of injections. B.) Animals sacrificed 14 days following the beginning of injections.

A.)



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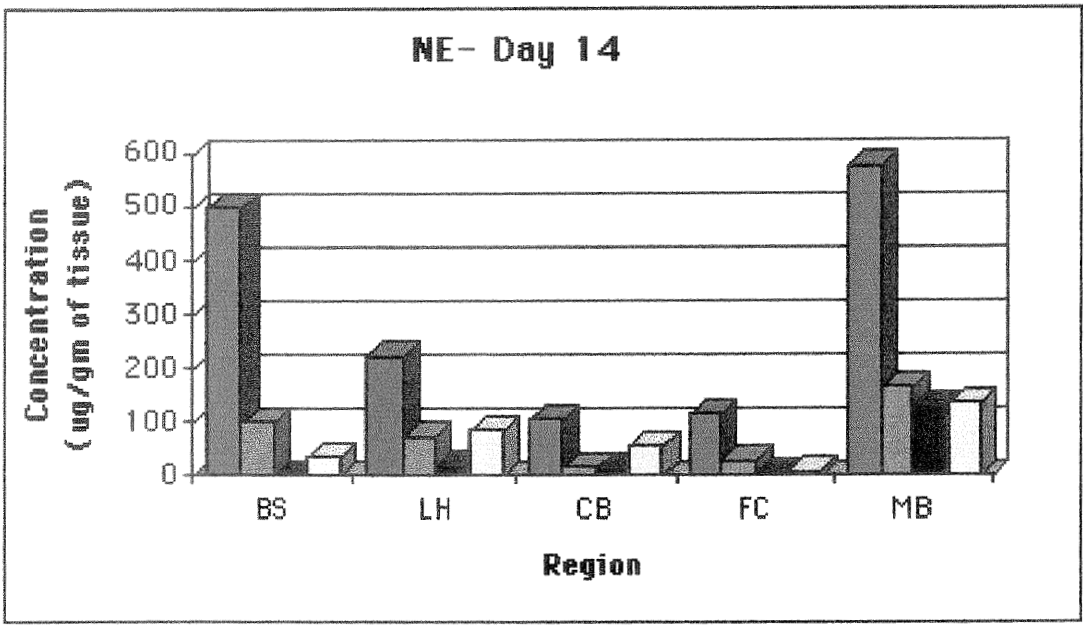
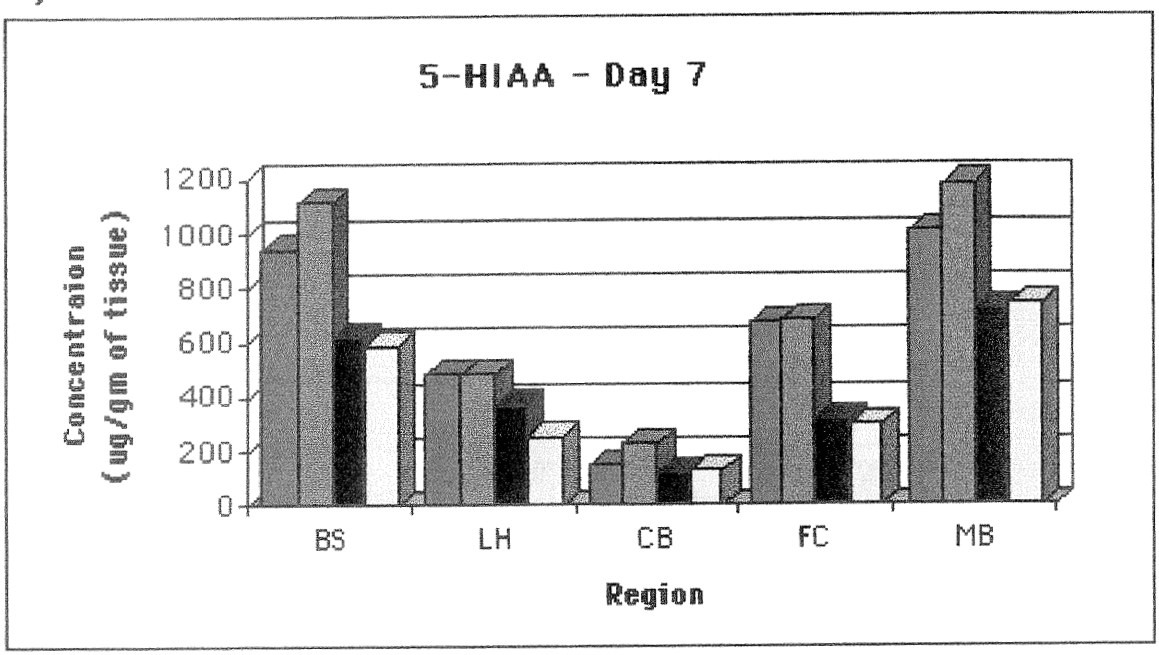


Figure 7. Average concentration of norepinephrine (NE) for each of the brain regions analyzed. BS= Brainstem, LH=Lateral Hemispheres CB=Cerebellum, FC=Frontal Cortex, and MB=Midbrain. A.) Animals sacrificed 7 days following the beginning of injections. B.) Animals sacrificed 14 days following the beginning of injections.

A.)



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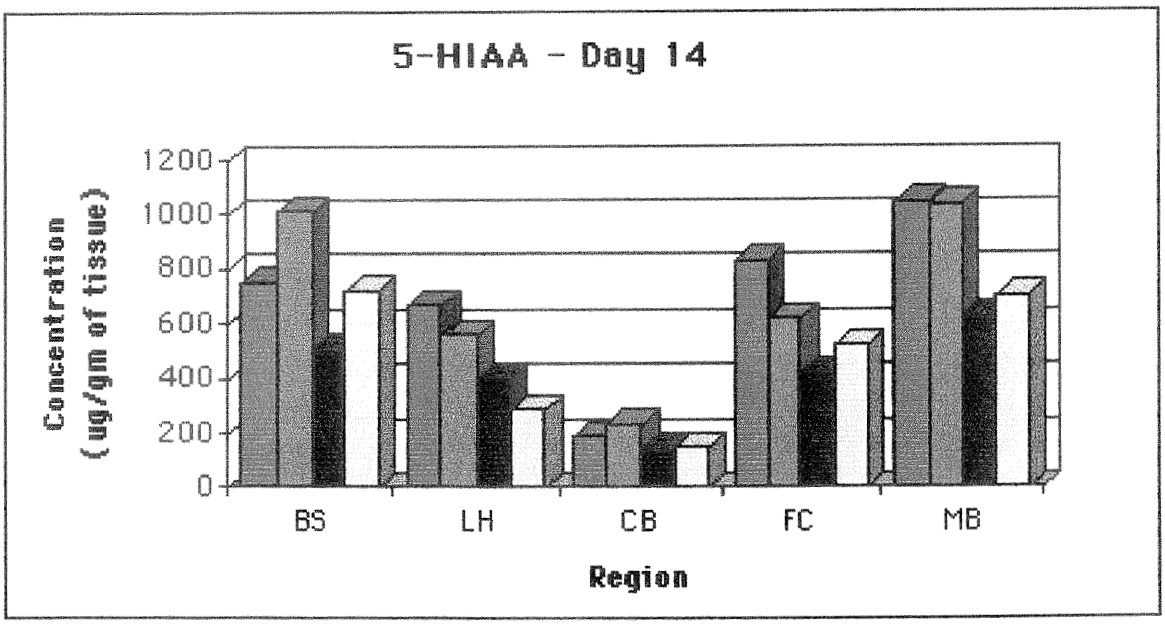
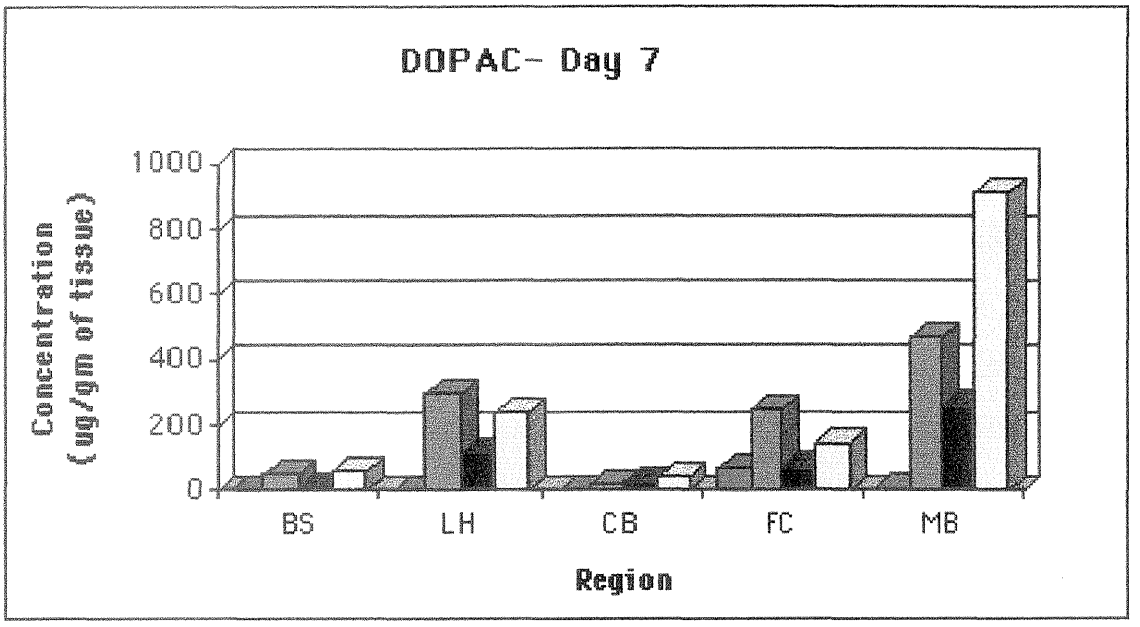


Figure 8. Average concentration of 5- HIAA for each of the brain regions analyzed. BS= Brainstem, LH=Lateral Hemispheres CB=Cerebellum, FC=Frontal Cortex, and MB=Midbrain. A.) Animals sacrificed 7 days following the beginning of injections. B.) Animals sacrificed 14 days following the beginning of injections.

A.)



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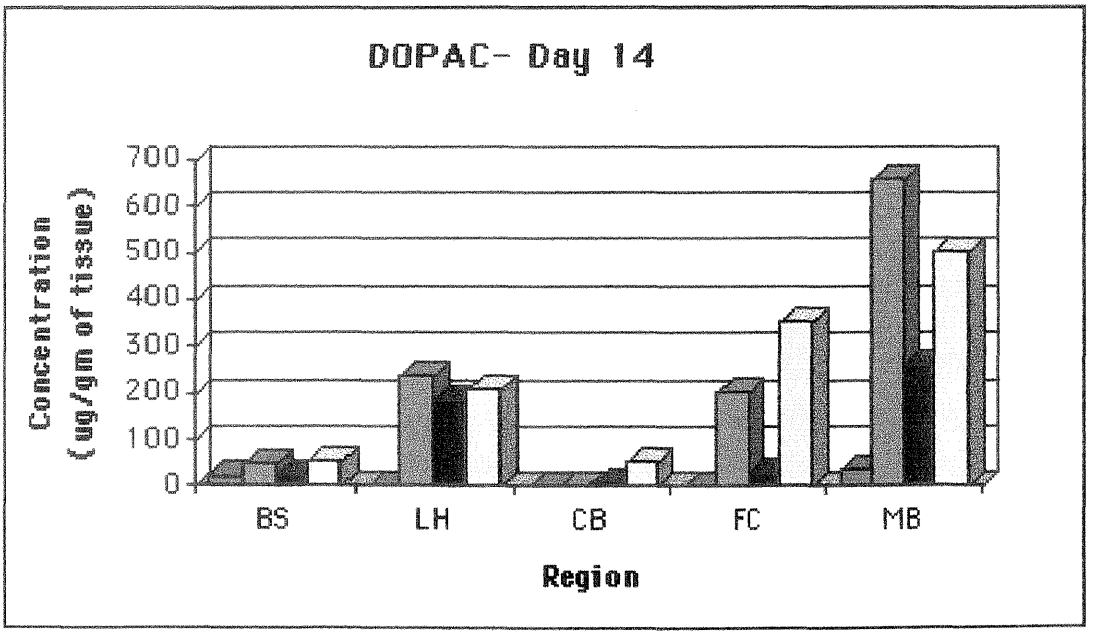

Figure 9. Average concentration of DOPAC for each of the brain regions analyzed. BS= Brainstem, LH=Lateral Hemispheres CB=Cerebellum, FC=Frontal Cortex, and MB=Midbrain. A.) Animals sacrificed 7 days following the beginning of injections. B.) Animals sacrificed 14 days following the beginning of injections.

There were no significant differences in the concentration of 5-HIAA between day 7 and day 14.

DOPAC (the metabolite of DA) concentrations found in each of the treatment groups were elevated over the concentrations in the control group and relatively consistent from day 7 to day 14. The most profound increase in DOPAC concentration was in the GM_1 and collateral treatment groups.

ANOVA and Tukey analysis of the biochemical data indicated that there were significant differences in the concentrations of the various substances examined, on a treatment and region dependent basis (see Tables 6-10).

The regions examined for 5-HT contained differing concentrations of the neurotransmitter depending on the treatment (see Table 6 and Figure 5). The concentration of 5-HT found in each of the regions from the day 7 and day 14 MDMA groups was found to be significantly different from controls by ANOVA and Tukey analysis. Serotonin concentrations were also significantly different in the MDMA + GM_1 groups. The GM_1 alone treatment produced concentrations of serotonin statistically different from controls in three instances: LH day 7, BS day 14, and CB day 14.

As can be seen in Table 7 and Figure 6, significantly different concentrations of dopamine (DA) were only found between the brainstems of day 7 controls and each of the day 7 treatment groups. With respect to norepinephrine concentrations, in all but three cases the concentration of the neurotransmitter was significantly different from controls. The three exceptions were the CB of GM_1 (day 7) animals, the CB of MDMA + GM_1 (day 14) animals, and the FC of GM_1 (day 14) animals (see Table 8 and Figure 7).

Significantly different concentrations of 5-HIAA were, in all but one case, found between GM_1 treated animals and other treatment groups. The concentrations of 5-HIAA found at day 7 post treatment in the brainstem of MDMA and MDMA + GM_1 treated animals were statistically different from the concentration found in the brainstem of GM_1

treated animals (Table 9). In the brainstem at day 14 post treatment, however, the only significant difference in 5-HIAA concentration was between the GM_1 treated animals and the MDMA treated animals. The cerebellums of the MDMA group and the MDMA + GM_1 group examined at day 7 and day 14 for 5-HIAA were found to contain significantly different concentrations of 5-HIAA relative to the concentration of 5-HIAA in the cerebellums of the GM_1 treated group. The concentration of 5-HIAA in the control group was statistically different from another group in only one case, and that was at day 14 post treatment in the lateral hemispheres of the MDMA + GM_1 treatment group.

Table 10 and Figure 9 show that midbrain concentrations of DOPAC were found to be statistically different between the controls (day 7) and the collateral treatment group (day 7).

DISCUSSION

As shown in Figure 3a, the activity of the animals at day five indicates that the MDMA alone treatment produced a significant hyperactivity relative to the control. The elevated level of activity observed in those animals treated with MDMA alone is a result consistent with the effects the neurotransmitter release induced by the recent administration of MDMA. Specifically, the serotonergic system has been implicated in exploratory behavior of rats (16), therefore, increasing the release of serotonin by treatment with MDMA should cause a corresponding increase in exploratory activity. The collateral treatment group (MDMA + GM_1), however, displayed a level of activity not significantly different from the level of activity displayed by the control group. Those animals treated with GM_1 alone also showed a significant increase in activity when compared to the control group.

An examination of the first five hours of activity at day 12 (Figure 4a), showed that the MDMA alone treated animals displayed a marked reduction in the level of activity relative to the controls. The reduced level of activity is entirely consistent with a depletion of neurotransmitters as a result of the long-term neurotoxic effects of chronic administration of MDMA. Decreased activity of the serotonergic system due to MDMA treatment might be directly related to a decrease in the exploratory behavior of the rats. At day 12 the collateral treatment group showed a level of activity above the control group. As at day five, the level of activity displayed by those animals treated with GM_1 alone, and examined at day 12, was markedly elevated relative to the controls.

In general, the level of activity was found to be much higher during the exploratory period of the animals at day 12 than during the exploratory period of the animals at day 5. The dissimilarity between the level of activity of the two groups of animals might be explained as being a difference in the disposition of the animals in each group.

The 12-hour light dark cycle was not reflected in the activity level of the animals (both day 7 and day 14). A possible explanation for this result could be that the animals were kept in a room that was adjacent to a larger area that housed dogs and the activity of the dogs may have affected the behavior of the rats.

The biochemical data shows that certain non-significant trends in neurotransmitter concentration predominate. Relative to the control groups, the concentration of each of the neurotransmitters examined was reduced in almost all of the tissues from animals treated with MDMA alone. The lower concentrations of neurotransmitters observed at day 7 are consistent with the depletion of neurotransmitters associated with the chronic phase (> 24 hours) of MDMA treatment (26). The reduced concentration of neurotransmitters at day 14 seems to be indicative of an insufficient amount of time to fully recover from the chronic effects of MDMA treatment. As the concentration of neurotransmitters found in the tissues taken from the collateral treatment group was consistently greater than the concentration of

the same neurotransmitters in the MDMA alone treatment group, it appears that GM_1 is exerting an attenuating effect on the neurotoxicity of MDMA treatment or enhancing the rate of recovery. Although the amount of neurotransmitters found in the tissues of the collateral treatment group were still lower than the levels observed in the control group, the amounts were, for the most part, between the control group and the MDMA treatment group. Those tissues taken from animals treated with GM_1 alone showed concentrations of neurotransmitters slightly lower than the controls in the majority of the tissues examined, a result which may be related to the neurotrophic actions of GM_1. Another explanation for the lower transmitter concentrations found for the GM_1 treatment group might be found in our method. The GM_1 tissues were processed at the beginning of the study and were temporarily stored in a -10° F freezer (a step that was not necessary with the other samples). The time in the freezer could have allowed degradation of the transmitter substance. To evaluate this possibility, the GM_1 biochemical analysis will be repeated.

ANOVA and Tukey analyses of the biochemistry data indicated that the concentrations of each of the neurotransmitters found in the tissues from the three treatment groups (MDMA, GM_1, and MDMA + GM_1) were, in general, significantly different from the concentrations of neurotransmitters found in the control tissues. These trends can be seen graphically in Figures 5-9.

The concentrations of serotonin, dopamine, and norepinephrine provides substantial evidence indicative of the trends outlined above. For example, the serotonin concentrations in tissues at day 7 and 14 (Table 6 and Figure 5) clearly follow these trends. Specifically, for the MDMA alone treated animals, the concentration of 5-HT was significantly lower than the concentration of 5-HT in the tissue samples collected from the control animals. A comparison of the concentrations of 5-HT between the tissues of the MDMA alone treated animals and the GM_1 alone treated animals showed a statistically insignificant trend: The concentration of 5-HT found in most of the tissues examined from

the MDMA treated animals was lower than the concentration of 5-HT found in the tissues taken from the MDMA + GM_1 treated animals. Furthermore, this trend can be seen at day 7 and day 14. Examining the data collected for NE and DA reveals similar trends. The similarities between the three neurotransmitters (5-HT, DA, and NE) are not surprising when one considers that one of the hypothesized modes of action by which MDMA produces its toxic effects is through an action on dopaminergic neurons which may then affect associated 5-HT and/or NE neurons.

There appears to be a trend in the concentrations of the neurotransmitters when the MDMA and MDMA + GM_1 treatments are compared at day 7 and day 14. Tables 6-8 show that both MDMA and MDMA + GM_1 treatments generally show an increase in the concentration of each of the neurotransmitters from day 7 to day 14. The MDMA + GM_1 group, however, appears to show a greater and more consistent increase from day 7 to day 14 than the increase seen in the MDMA treatment group. That this trend exists would seem to suggest that the GM_1 collateral treatment may enhance the rate of recovery from MDMA-induced toxicity with respect to the neurotransmitters that were examined.

Two general observations can be made with respect to the metabolites of 5-HT (5-HIAA) and DA (DOPAC). Treatment with MDMA or the collateral treatment decreased the concentration of 5-HIAA but increased the concentration of DOPAC in the tissues. The reasons for these trends is not clear, but may be related to the selectively serotonergic toxicity of MDMA: MDMA causes damage to serotonergic fibers, decreasing serotonin and 5-HIAA concentrations, but not to dopaminergic fibers, allowing increased release of DA and a subsequent increase in DOPAC concentration.

The treatment regimen followed in this experiment was dictated by dosages employed in other studies (2, 3, 4, 14, 20, 26, 31, 32, 33, 34, 38, 44). MDMA was administered in four doses in order to deliver a sufficient amount of the drug to induce toxicity while not overtly harming the animals. The amount of GM_1 administered (40

mg/kg) reflected dosages used in other studies (10, 19). Alteration of the treatment regimen could have an appreciable effect on the results of the study. Although insignificant trends were observed, a change in the treatment regimen (such as increasing the amount of GM_1 administered) may produce significant results in future trials.

CONCLUSION

The behavioral data suggests that animals recover more quickly from the behavioral effects of MDMA when given a collateral administration of GM_1. The biochemical data (although, admittedly, not as conclusive as the behavioral data) shows trends indicative of an enhanced rate of recovery from MDMA-induced toxicity in those animals that received collateral treatment with GM_1. Due to the nature of our results, it would seem safe to conclude that co-administration of GM_1 has an effect on the behavior of animals also treated with MDMA. However, a biochemical explanation as to the nature of that effect is still unclear. The results of this experiment indicate that further work is merited.

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Appendix I

Behavioral Activity
Day 5

Table A1. Mean +- standard deviation for each hour of the 24-hour period of the day 5 behavior activity study

	DAY 5			
TIME	Control	GM1	MDMA	MDMA + GM1
13:00	321.2 ± 275.8	588.6 ± 339.5	514.2 ± 149.8	284.4 ± 118.2
14:00	141.8 ± 253.1	357.2 ± 277.3	531.8 ± 225.2	200 ± 180.6
15:00	33.8 ± 47.2	347.2 ± 315.2	445.8 ± 218.9	103.8 ± 39.4
16:00	15.8 ± 15.7	225.4 ± 241.0	167 ± 171.9	86.8 ± 47.9
17:00	29.8 ± 51.1	150.6 ± 107.4	239.8 ± 251.8	70 ± 14.5
18:00	24.8 ± 38.5	229.2 ± 151.6	325.6 ± 443.2	74.6 ± 65.4
19:00	25.8 ±23.8	157 ± 190.1	172.2 ± 82.8	105.2 ± 76.6
20:00	39.6 ± 57.5	100.8 ± 80.4	201.8 ± 106.9	90.8 ± 55.7
21:00	12.6 ± 17.5	151.4 ± 152.8	237.2 ± 287.9	59 ± 11.9
22:00	2.2 ± 0.8	109.6 ± 58.1	118.2 ± 6.1	94.6 ± 101.8
23:00	4.2 ± 4.5	199.8 ± 112.5	266.2 ± 284.0	93.2 ± 107.6
0:00	16.4 ± 22.6	153.2 ± 118.5	50.6 ± 22.1	79.4 ± 90.9
1:00	12.8 ± 10.8	156.4 ± 154.9	180.4 ± 158.5	153.2 ± 181.1
2:00	55 ± 90.6	305.8 ± 226.5	296.4 ± 355.5	72.6 ± 17.7
3:00	30.8 ± 36.8	57.8 ± 48.8	103.6 ± 147.6	194.6 ± 152.0
4:00	57.2 ± 65.7	71.6 ± 52.0	162.8 ± 147.0	57 ± 55.5
5:00	27.4 ± 34.4	68.4 ± 37.7	236.4 ± 244.0	99.4 ± 90.9
6:00	47.4 ± 55.4	141 ± 177.7	122 ± 177.1	44.8 ± 46.2
7:00	65.6 ± 60.4	173.6 ± 177.0	254 ± 242.9	61 ± 27.8
8:00	121 ± 209.5	184.8 ± 171.5	252 ± 188.8	119.4 ± 180.9
9:00	39.6 ± 39.3	43 ± 44.6	118.2 ± 126.7	92 ± 61.4
10:00	33 ± 24.9	33.6 ± 20.1	244.8 ± 312.5	118.8 ± 136.1
11:00	65.6 ± 82.2	155.6 ± 148.0	190.4 ± 179.9	297.6 ± 308.9
12:00	120.8 ± 241.3	31.6 ± 18.5	129.4 ± 101.9	110 ± 101.7

Appendix II

Behavioral Activity
Day 12

Table A2. Mean +- standard deviation for each hour of the 24-hour period of the day 12 behavior activity study

	Day 12			
Time	Control	GM1	MDMA	MDMA + GM1
13:00	1040.2 ± 576.6	1452.4 ± 698.4	271.6 ± 331.3	1004.4 ± 342.3
14:00	663.2 ± 462.5	1083.6 ± 483.3	162.6 ± 68.2	874 ± 469.2
15:00	532 ± 511.9	823 ± 337.6	150 ± 53.2	903.2 ± 713.8
16:00	220.2 ± 198.5	395.4 ± 485.4	137.8 ± 65.5	601.2 ± 196.3
17:00	146.4 ± 63.5	378 ± 243.4	179.4 ± 112.8	212.6 ± 136.2
18:00	217.2 ± 81.8	112 ± 63.4	216.2 ± 178.7	227.4 ± 130.7
19:00	203.4 ± 254.3	178 ± 114.5	395.4 ± 352.7	150.6 ± 178.5
20:00	306.4 ± 295.6	266.6 ± 255.5	246.6 ± 289.2	208.8 ± 102.8
21:00	210.8 ± 152.4	122.4 ± 61.9	186.6 ± 75.2	140.8 ± 164.1
22:00	139.6 ± 124.6	75.2 ± 74.8	119.2 ± 78.5	93 ± 48.2
23:00	147.2 ± 261.9	141.6 ± 111.5	231.4 ± 195.3	46 ± 12.8
0:00	102.2 ± 104	113.4 ± 46.4	188.6 ± 139.4	176.8 ± 104.1
1:00	140.8 ± 83.9	80.6 ± 43.3	171.6 ± 149.9	124.2 ± 93.0
2:00	113.8 ± 98.2	104.2 ± 87.6	149 ± 90.9	268.4 ± 287.8
3:00	166.8 ± 155.5	50.6 ± 13.6	289.6 ± 291.5	102.8 ± 25.6
4:00	237.2 ± 458.8	67.6 ± 39.8	179.6 ± 158.5	108.2 ± 68.6
5:00	57.2 ± 44.7	120.6 ± 84.4	45.6 ± 44.6	190.8 ± 182.5
6:00	19.4 ± 17.7	113.6 ± 90.2	58.2 ± 43.2	248 ± 130.6
7:00	183.4 ± 245.1	53.2 ± 37.5	61.6 ± 75.0	276.8 ± 268.1
8:00	128 ± 168.5	72.4 ± 49.7	57.4 ± 64.8	233.4 ± 156.3
9:00	116.4 ± 125.8	152.4 ± 135.7	55.2 ± 51.0	78.2 ± 38.9
10:00	75.8 ± 8.9	263.2 ± 382.0	28.8 ± 19.9	214.8 ± 107.7
11:00	115.6 ± 155.2	109 ± 94.1	14.6 ± 13.1	287 ± 146.2
12:00	173.6 ± 134.6	134.4 ± 149.7	39.8 ± 51.9	244.2 ± 148.3